EXPRESS MAIL CERTIFICATE

DOCKET NO.: **19603/2350 (CRF D-1510B)**

APPLICANT: **Erik Falck-Pedersen**

TITLE: **ADENOVIRUS GENE EXPRESSION SYSTEM**

Certificate is attached to the **Declaration of Erik S. Falck-Pedersen Under 37 CFR § 1.132 (4 pages)** of the above-named application.

EXPRESS MAIL NUMBER: **EM009597869US**

DATE OF DEPOSIT: **June 25, 1998**

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231, Box: CPA.

Wendy L. Harrold
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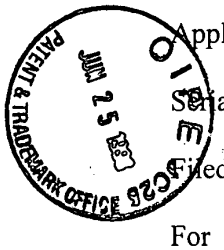
Wendy L. Harrold

(Signature of person mailing paper or fee)

PATENT
19603/2350 (CRF D-1510B)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE #23



Applicant	:	Erik Falck-Pedersen	Examiner: B. Campell
Serial No.	:	CPA of 08/653,114	
Filed	:	---	Art Unit: 1819
For	:	ADENOVIRUS GENE EXPRESSION SYSTEM	

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DECLARATION OF ERIK S. FALCK-PEDERSEN UNDER 37 CFR § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, ERIK S. FALCK-PEDERSEN, pursuant to 37 CFR § 1.132, declare:

1. I received a B.A. in Chemistry/Biology in 1976 from North Central College and a Ph.D. in 1981 in Biochemistry from University of Illinois.

2. I am a Professor, Department of Microbiology, Cornell University Medical College.

3. I am the named inventor of the above-identified patent application.

4. I am familiar with disclosures of Quantin, et al., "Adenovirus as an Expression Vector in Muscle Cells *in vivo*," Proc. Nat'l. Acad. Sci. USA, 89:2581-84 (1992) ("Quantin"), Stratford-Perricaudet, et al., "Widespread Long-term Gene Transfer to Mouse Skeletal Muscles and Heart," J. Clin. Invest., 90:626-30 (1992) ("Stratford-Perricaudet"), Kirshenbaum, et al., "Highly Efficient Gene Transfer into Adult Ventricular Myocytes by Recombinant Adenovirus" J. Clin. Invest., 92:381-87 (1993) ("Kirshenbaum"), and Huang, et al., "Intervening Sequences Increase Efficiency of RNA 3' Processing and Accumulation of Cytoplasmic RNA," Nucl. Acids Res., 18(4):937-47 (1990) ("Huang"). I am submitting this declaration to demonstrate that the vector which constitutes the subject matter of my present invention is patentable over these references.

5. Kirshenbaum discloses a recombinant adenovirus vector for gene transfer into ventricular myocytes. This vector utilizes, in order, part of the adenovirus type 5

genome, a human cytomegalovirus immediate-early promoter, an *E. coli* lacZ gene, an SV40 polyadenylation signal, and part of the adenovirus type 5 genome.

6. Quantin discloses a recombinant adenovirus expression vector for gene transfer to muscle cells. This vector contains, in order, a portion of the adenovirus 5 genome, an enhancer fragment of the mouse myosin light chain 1/3 locus, the mouse skeletal α-actin gene promoter, the β-galactosidase gene, the polyadenylation segment from simian virus, and part of the adenovirus 5 genome.

7. Stratford-Perricaudet discloses a recombinant adenovirus expression vector for gene transfer to mouse skeletal muscles and heart. In sequence, this vector includes a portion of an adenovirus 5 genome, the Rous sarcoma virus long terminal repeat, the β-galactosidase gene, the polyadenylation sequence from simian virus 40 early region, and part of the adenovirus genome 5.

8. Thus, neither Quantin, Stratford-Perricaudet, nor Kirshenbaum disclose utilizing a splice acceptor and splice donor site in their vectors.

9. Huang discloses expression vectors for gene transfer into mammalian cells. The pMLSS.CAT vector includes, in series, a simian virus 40 early promoter with enhancer, an adenovirus major late region, an IgG variable region which forms a splice acceptor region, a chloramphenicol acetyl transferase encoding gene, and a late polyadenylation site. There are no left end replication and package elements of the adenovirus 5 genome upstream of the promoter nor an adenovirus 5 genome region positioned downstream of an insertion site. Accordingly, the vector of Huang is not capable of forming a recombinant adenovirus.

10. The following experimental work is submitted to demonstrate that the subject matter of my present invention is patentable over Quantin, Stratford-Perricaudet, Kirshenbaum, and Huang.

11. Vectors pMLSISCAT, pMLSISCATL3dISS, pMLSISCATL3, pMLSISCATgD, and pAdCMVCATgD were constructed as shown below in Figure 1.

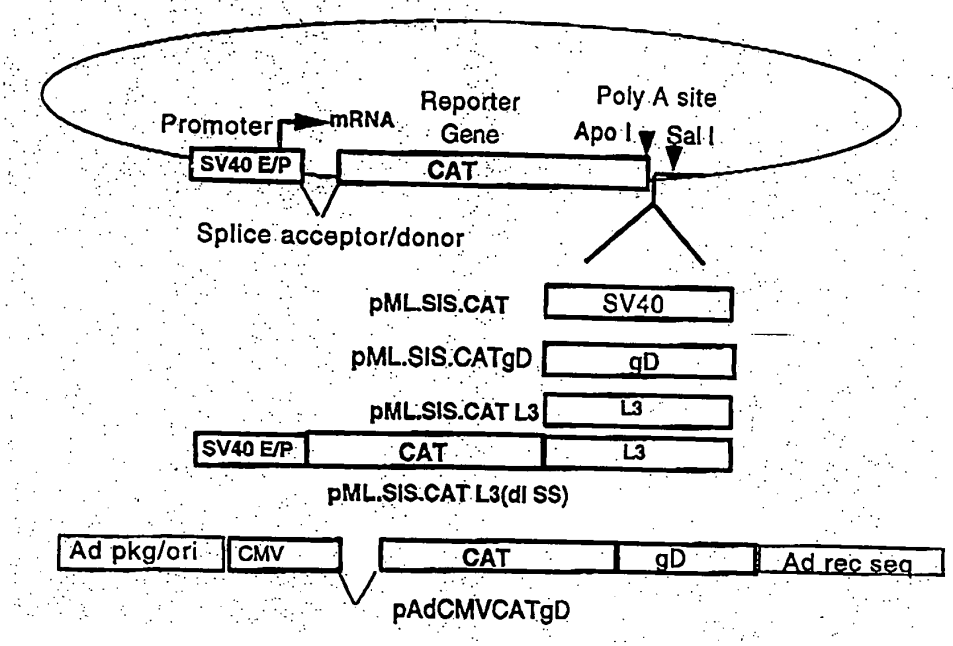

FIGURE 1

To compare their relative expression levels, the vectors were transfected into 293 human embryonic kidney cells. Forty-eight hours after transfection, cells were harvested, and lysates were prepared and assayed for CAT enzymatic activity. In this assay, high expression level is indicated by increased production of acetylated products. The results of this experimental work are shown below in Figure 2.

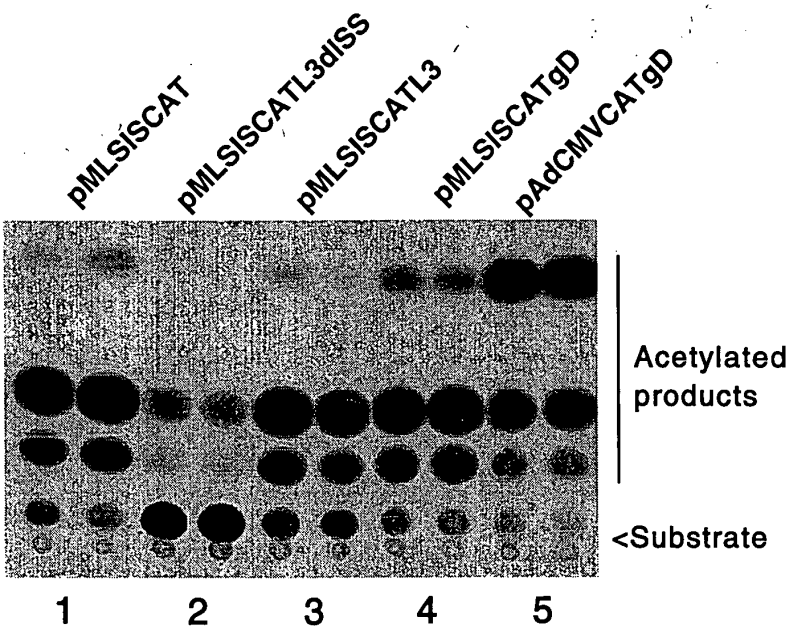

FIGURE 2

12. A comparison of the results in Figure 2 for the pMLSISCATL3dISS vector versus those achieved with the other vectors tested demonstrates that substantially greater amounts of acetylated product are achieved when the vector has a splice donor and splice acceptor site. Thus, the vector of my present invention achieves significantly better expression than do the vectors of Quantin, Stratford-Perricaudet, or Kirshenbaum which lack such a site.

13. As to the significance of utilizing the vector of my present invention over that of Huang, a comparison of the results in Figure 2 for the pMLSISCAT vector versus the results for the pAdCMVCATgD vector shows that much higher levels of expression are achieved with my present invention than with Huang's vector.

14. Thus, my above-described experimental work shows that the subject matter of my present invention achieves substantial greater expression levels than any of Quantin, Stratford-Perricaudet, Kirschenbaum, or Huang.

15. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 6/18/98

Erik S. Falck-Pedersen